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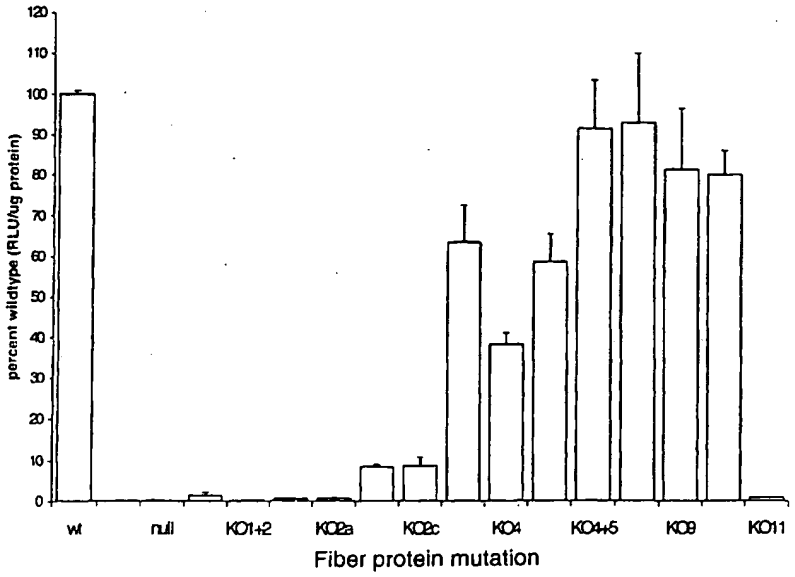
[Continued on next page]

(54) Title: ADENOVIRUS PARTICLES WITH MUTAGENIZED FIBER PROTEINS



(57) Abstract: This invention relates to mutated adenoviral fiber proteins and adenovirus particles containing such proteins. It further relates to polynucleotides encoding the proteins and vectors containing polynucleotides. It also relates to methods for making and using the adenoviral particles. With the mutated fiber proteins, the adenovirus particles no longer bind to their natural cellular receptor. They can then be "retargeted" to a specific cell type through the addition of a ligand to the virus capsid, which causes the virus to bind to and infect such cell. Specific fiber mutations are listed, which ablate binding to the natural receptor. Adenovirus particles with certain fiber mutations were found to enhance gene transfer to and expression in liver as compared to viral particles with wild-type fiber.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/86 C12N15/34 C12N5/10 C07K14/075 C12N7/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
SEQUENCE SEARCH, CHEM ABS Data, EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 15823 A (GENVEC INC) 23 March 2000 (2000-03-23) claims 4,5; tables 1,2 ---	1-6, 8-11, 13-16, 18-32, 52-54, 59,61,62
X	WO 98 44121 A (MEHTALI MAJID ;LEGRAND VALERIE (FR); TRANSGENE SA (FR); BOULANGER) 8 October 1998 (1998-10-08) claims 4,8-10 --- -/--	1-5, 8-11, 13-16, 18-32, 52-54, 59,61,62

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 April 2003	01 07 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 50053 A (GENETIC THERAPY INC ;STEVENSON SUSAN C (US); GORZIGLIA MARIO (US);) 12 November 1998 (1998-11-12) ---	
A	WO 98 07865 A (GENVEC INC) 26 February 1998 (1998-02-26) -----	

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WO 0015823 A	23-03-2000	AU 5819499 A	03-04-2000
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 01/06286

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 27-30, 45-48, and 50-58, as far as they relate to in vivo methods, are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-6, 8-11, 13-16, 18-32, 52-54, 59, 61, 62 partially

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-6, 8-11, 13-16, 18-32, 52-54, 59, 61, 62 (all partially)

1.1. Claims: 1-6, 8-11, 13-16, 18-32, 52-54, 59, 61, 62 (all partially)

A mutated adenoviral fiber protein wherein at least aa 441 has been mutated; polynucleotide encoding said fiber; adenoviral particle comprising said fiber; methods and compositions relating thereto.

1.2. Claims: 1-6, 8-11, 13-16, 18-32, 52-54, 59, 61, 62 (all partially)

A mutated adenoviral fiber protein wherein at least aa 442 has been mutated; polynucleotide encoding said fiber; adenoviral particle comprising said fiber; methods and compositions relating thereto.

2. Claims: 7, 12, 17, 21-23 (all partially)

A mutated adenovirus serotype 5 fiber protein comprising at least a mutation at amino acid 460.

3. Claims: 7, 12, 17, 21-23 (all partially)

A mutated adenovirus serotype 5 fiber protein comprising a at least mutation at amino acid 509.

4. Claims: 7, 12, 17, 21-23 (all partially)

A mutated adenovirus serotype 5 fiber protein comprising a at least mutation at amino acid 510.

5. Claims: 7, 12, 17, 21-23 (all partially)

A mutated adenovirus serotype 5 fiber protein comprising a at least a mutation at amino acid 538.

6. Claims: 7, 12, 17, 21-23 (all partially)

A mutated adenovirus serotype 5 fiber protein comprising a at least mutation at amino acid 539.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. Claims: 33-51, 55-58, 60 (all totally) and 52-54, 61, 62 (all partially)

A mutated adenovirus serotype 5 fiber protein comprising mutations at amino acid positions 408 and 409; polynucleotide encoding said fiber; adenoviral particle comprising said fiber; methods and compositions relating thereto.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

ADENOVIRUS PARTICLES WITH MUTAGENIZED FIBER PROTEINS

Claims of **WO0192299**

CLAIMS:1. A mutated adenoviral fiber protein wherein at least one amino acid in the CD loop of a wild-type fiber protein of an adenovirus from subgroup C, D, or E, or the long wild-type fiber of an adenovirus from subgroup F, has been mutated to reduce or substantially eliminate the ability of said fiber protein to bind to the coxsackievirus-adenovirus receptor (CAR).

2. The mutated adenoviral fiber protein of claim 1, wherein said mutation substantially eliminates the ability of said protein to bind to said CAR.

3. The mutated adenoviral fiber protein of claim 2, wherein said fiber protein is an adenovirus serotype 5 fiber protein.

4. The mutated adenoviral fiber protein of claim 3, wherein said fiber protein contains at least one mutation at amino acid positions 441 and 442 of the wild-type fiber protein.

5. The mutated adenoviral fiber protein of claim 4, wherein said fiber protein further comprises a mutation at one or more of the following amino acid positions of the wild type fiber protein: 408,409,460,509,510,538, and 539.

6. The mutated adenoviral fiber protein of claim 4, wherein said fiber protein further comprises at least one mutation at amino acid positions 408 and 409 of the wild-type fiber protein.

7. A mutated adenovirus serotype 5 fiber protein wherein said fiber protein comprises a mutation at one or more of the following amino acid positions of the wild-type fiber protein: 460,509,510,538, and 539, wherein said mutation reduces or substantially eliminates the ability of said fiber protein to bind to CAR.

8. A polynucleotide encoding the protein of claim 1.

9. A polynucleotide encoding the protein of claim 3.

10. A polynucleotide encoding the protein of claim 4.

11. A polynucleotide encoding the protein of claim 5.

12. A polynucleotide encoding the protein of claim 7.

13. An adenoviral particle comprising the fiber protein of claim1.

14. An adenoviral particle comprising the fiber protein of claim 3.

15. An adenoviral particle comprising the fiber protein of claim 4.

16. An adenoviral particle comprising the fiber protein of claim5.

17. An adenoviral particle comprising the fiber protein of claim 7.

18. The adenoviral particle of claim 13 further comprising a targeting ligand included in a capsid protein of said particle.

19. The adenoviral particles of claim 18 wherein said capsid protein is the mutated adenoviral fiber protein.

20. The adenoviral particle of claim 19 further comprising at least one heterologous polynucleotide.

21. The adenoviral particle of any one of claims 14-17 further comprising a targeting ligand included in a capsid protein of said particle.

22. The adenoviral particle of claim 21 wherein said capsid protein is the mutated adenoviral fiber protein.

23. The adenoviral particle of claim 22 further comprising at least one heterologous polynucleotide.

24. An adenovirus packaging cell comprising the polynucleotide of claim 8.

25. A method of making the adenoviral particle of claim 13, comprising the stepsof : transferring the adenovirus genome to be packaged in said particle into the packaging cell of claim 24; culturing said packaging cell; and recovering an adenoviral particle produced by said cell.

26. A method of making the adenoviral particle of claim 18 comprising the stepsof : transferring the adenovirus genome to be packaged in said particle into a cell having adenovirus polynucleotides that provide proteins necessary for the replication, maturation, and packaging of said genome; culturing said cell under conditions permitting the production of said particle; and recovering an adenoviral particle produced by said cell.

27. A method of expressing a heterologous polynucleotide in a cell comprising infecting said cell with the adenoviral particle of claim 20.

28. The method of claim 27, wherein said cell is a mammalian cell.

29. The method of claim 28, wherein said mammalian cell is a primate cell.

30. The method of claim 29, wherein said primate cell is a human cell.

31. A composition comprising the adenoviral particle of claim 18 in a pharmaceutically acceptable carrier.

32. A composition comprising the adenoviral particle of claim 20 in a pharmaceutically acceptable carrier.

33. A mutated adenovirus serotype 5 fiber protein wherein said fiber protein contains mutations at amino acid positions 408 and 409 of the wild-type fiber protein.

34. The mutated fiber protein of claim 33, wherein said protein contains deletions at amino acid positions 408 and 409 of the wild-type fiber protein.

35. The mutated fiber protein of claim 33, wherein said protein contains amino acid substitutions at amino acid positions 408 and 409 of the wild-type fiber protein.

36. The mutated fiber protein of claim 35, wherein glutamic acid is substituted for serine at position 408 and alanine is substituted for proline at position 409 (SEQ ID NO: 4).

37. A polynucleotide encoding the protein of claims 33-36.

38. An adenoviral particle comprising the fiber protein of claims 33-36.

39. The adenoviral particle of claim 38, further comprising a targeting ligand included in a capsid protein of said particle.

40. The adenoviral particle of claim 39, further comprising at least one heterologous polynucleotide.

41. The adenoviral particle of claim38, wherein at least one of the penton proteins of said particle has been modified to delete the RGD sequence.

42. An adenovirus packaging cell comprising the polynucleotide of claim 37.

43. A method of making the adenoviral particle of claim 38, comprising the steps of : transferring the adenovirus genome to be packaged in said particle into the packaging cell of claim 42; culturing said packaging cell; and recovering an adenoviral particle produced by said cell.

44. A method of making the adenoviral particle of claim 38, comprising the stepsof : transferring the

adenovirus genome to be packaged in said particle into a cell having adenovirus polynucleotides that provide proteins necessary for the replication, maturation, and packaging of said genome; culturing said cell under conditions permitting the production of said particle ; and recovering an adenoviral particle produced by said cell.

45. A method of expressing a heterologous polynucleotide in a cell comprising infecting said cell with the adenoviral particle of claim 40.

46. The method of claim 45, wherein said cell is a mammalian cell.

47. The method of claim 45, wherein said cell is a primate cell.

48. The method of claim 45, wherein said cell is a human cell.

49. A composition comprising the adenoviral particle of claim 40 in a pharmaceutically acceptable carrier.

50. A method of enhancing adenoviral-mediated gene transfer to and expression in hepatocytes comprising the steps of administering adenoviral particles of claim 40 to said hepatocytes.

51. A method of enhancing adenoviral-mediated gene transfer to and expression in hepatocytes comprising the stepsof : preparing an adenovirus particle comprising a mutated adenovirus serotype 5 fiber protein, wherein glutamic acid is substituted for serine at amino acid position 408 and alanine is substituted for proline at amino acid position 409, and further comprising a heterologous gene; and infecting hepatocytes with said adenovirus particle.

52. A method of expressing a protein in a mammal comprising the step of administering the adenoviral particle of claim 20 or claim 40 to said mammal, wherein said particle transduces a cell in said mammal and said heterologous polynucleotide expresses said protein in said cell.

53. The method of claim 52, wherein said mammal is a primate.

54. The method of claim 53, wherein said primate is a human.

55. A method of expressing a protein in the liver of a mammal comprising administering a sufficient amount of the adenoviral particles of claim 40 for said particles to transduce cells in the liver of said mammal.

56. The method of claim 55, wherein said amount comprises approximately 1 particle per kilogram of body weight to approximately1013 particles per kilogram of body weight.

57. The method of claim 55, wherein said amount comprises approximately104 particles per kilogram of body weight to approximately1012 particles per kilogram of body weight.

58. The method of claim 55, wherein said amount comprises approximately108 particles per kilogram of body weight to approximately 1011 particles per kilogram of body weight.

59. An adenoviral vector comprising the polynucleotide of any one of claims 8-12.

60. An adenoviral vector comprising the polynucleotide of claim 37.

61. The adenoviral particle of claims 13,18,20,38,39,40, or 41, wherein said adenoviral particle is a replication conditional adenovirus.

62. The adenoviral particle of claim 61, wherein said adenovirus is an oncolytic adenovirus.